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Year: 2020

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DOI: https://doi.org/10.1126/science.abb8577

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ZORA URL: https://doi.org/10.5167/uzh-191060
Journal Article
Accepted Version

Originally published at:
Aguzzi, Adriano; De Cecco, Elena (2020). Shifts and drifts in prion science. Science, 370(6512):32-34.
DOI: https://doi.org/10.1126/science.abb8577

Shifts and Drifts in Prion Science

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***Summary sentence*: Our understanding of prions has progressed spectacularly since their discovery four decades ago, yet some of the most important questions remain open.**

Paradigm shifts are drivers of scientific progress, yet the shifters of the paradigms often experience scorn rather than immediate applause. That was the fate of Stanley Prusiner's 1982 paper claiming - to the initial amusement of his colleagues - that scrapie, a degenerative disease that affects the central nervous system of sheep, is caused by "proteinaceous infectious particles", which he termed prions (*1*). Prusiner's intuition, which earned him the 1997 Nobel Prize, is influencing our approach to an ever-expanding variety of seemingly unrelated diseases and physiological processes, and its implications reverberate to the present day.

The two decades preceding Prusiner's paper had witnessed the immense success of molecular biology, including the cracking of the genetic code, the elucidation of DNA replication, transcription and translation, and the cloning of genes. These discoveries prompted Francis Crick to conceptualize the "Central Dogma": information flows unidirectionally from DNA to proteins. But while religious dogmas may be eternal, the shelf life of scientific dogmas is inevitably limited. Prusiner postulated that prions carry on their replicative cycle without the participation of nucleic acids. This hypothesis, reminiscent of Griffith's 1967 suggestion of the existence of self-replicating proteins

(*2*), had the potential to explain the prodigious resistance of the scrapie agent to DNA-damaging radiation. Carleton Gajdusek, who won a Nobel Prize for showing that Kuru was a human disease transmitted by cannibalism in Papua New Guinea, had proposed in 1959 that the neurodegenerative disorders Kuru, scrapie and Creutzfeldt-Jakob disease (CJD) are caused by "slow viruses". Indeed, prions behave similarly to neurotropic viruses in many surprising ways, including the colonization of extraneural organs followed by neuroinvasion of the brain via peripheral nerves (*3*). Yet Prusiner purified the agent and found it to be smaller than a virus: no informational nucleic acid would fit into it. Over time, the group of prion diseases grew to include other human (Fatal Familial Insomnia) and animal disorders (Mad Cow Disease and Chronic Wasting Disease), but no causative virus has been identified and their prion etiology is now well accepted.

But prions did not contradict Crick's Central Dogma after all. Charles Weissmann, refusing to believe that a protein could exist without its respective gene, discovered in hamsters the gene encoding the cellular prion protein (PrP^{C}), whose misfolding yields tightly-packed aggregates called PrP^{Sc}. It is generally believed that prion replication occurs when coalesced PrP^{Sc} is broken down into smaller species. Those species then accrue further PrP^{Sc} in a process akin to the growth of crystals – and eventually break again, perpetuating their replicative cycle. Infectious prion seeds then move to neighboring cells and wreak havoc in the central nervous system by inducing vacuolation ("spongiosis") within neurons.

Does this mean that PrP^{Sc} is the prion? Weissmann's discovery in 1993 that *Prnp*-ablated mice are resistant to scrapie (*4*) was designed to disprove the protein-only hypothesis, and failed to do so - but it fell short of proving it. If PrP^{C} were the receptor of an imaginary "scrapie virus", its ablation may also render mice resistant to scrapie. More direct evidence for Prusiner's ideas emerged in 2001 from Claudio Soto's landmark experiment: repeated cycles of PrP^{Sc} fragmentation, when followed by addition of PrP^{C} and aggregate regeneration, can multiply prions ad libitum (*5*). These

findings strengthen the hypothesis that transfer of structural information can occur horizontally between proteins.

More recently the prion concept has been applied, sometimes overenthusiastically, to virtually all diseases characterized by progressive deposition of aggregated proteins in the central nervous system, whether infectious or not – and even to physiological processes such as memory formation (*6*). α-synuclein aggregates can self-propagate in the brains of Parkinson's patients (*7*), in cultured cells and in mice (*8*). This implies that synuclein is a de facto prion, and that its handling demands high biosafety standards. Similar arguments were made for tau and Aβ aggregates, the major hallmarks of Alzheimer's disease (*9*). However, prions caused many epidemics, whereas infectiousness has not been conclusively demonstrated for other protein aggregates, and specifically not via oral transmission Protein aggregates that were not shown to be serially transmissible across multiple generations of hosts are better regarded as "prionoids", even if they share molecular mechanisms of amplification with bona fide prions *in vitro*.

As predicted by Prusiner in the closing lines of his paper, the "prion revolution" boosted research in the field of neurodegeneration by providing an intellectual framework that might explain many aspects of Alzheimer's, Parkinson's and many other diseases featuring protein aggregates. While cellular PrP^{C} is now known to be crucial for the maintenance of peripheral myelin (*10*), our understanding of prions has essentially stagnated for more than a decade, and may now be lagging behind that of prionoids. What do we really know about prions, after almost 40 years from Prusiner's discovery?

One crucial obstacle to advancing prion research is the lack of high-resolution structures of PrP^{Sc} due to its insolubility, its non-crystalline aggregational state, and the persistent difficulties in preparing high-purity infectious material *de novo* from recombinant protein (*11*). This raises the possibility that infectious aggregates may constitute a sparsely-populated conformational variant

within such preparations. If so, most material aggregated *in vitro* may be non-infectious, and may not be informative of the structure of the prion and of its replicative mechanism.

Among all the models that have been proposed so far, the most plausible suggests that the prion consists of fibrils arranged as four-rung β-solenoids (*12*) stacked either head-to-tail or head-to-head. Cryo-electron microscopy of purified glycosylphosphatidylinositol (GPI)-anchorless prion fibrils (*13*) supports this model, thus providing the first high-magnification images of infectious prions, albeit the resolution does not suffice to determine the precise arrangement of the monomers within the fibrils. These structures are quite different from those of tau, α–synuclein and Aβ, and differ also from recombinant PrP fibrils – all of which are arranged in long fibers with no cavity. Hence PrP^{Sc} has unique structural characteristics, but it is unknown whether and how these peculiarities relate to their frightening infectivity.

The link between the generation of PrP aggregates and their neurotoxicity is also unclear. A large body of evidence (*14*) indicates that PrP^{C} is necessary for toxicity, perhaps because extracellular PrP^{Sc} oligomers dock to PrP^{C} on cell surfaces. Another aspect unique to prion infections pertains to the peculiar morphology of the damage that it wreaks on the brain. Of all aggregation-prone proteins, prions are the only ones that cause extensive intraneuronal vacuolation (spongiosis), whose severity increases during disease progression. This phenomenon is as much intriguing as it is mysterious. To date, almost nothing is known about the cellular and molecular pathology underlying vacuole formation; yet its ubiquity in all known prion diseases suggests that they are a prime driver of toxicity – and therefore also a target for therapeutic interventions.

High-resolution 3D structures of prions are also required to solve the long-standing question of prion strains, which share the same PrP sequence and yet cause distinct diseases (e.g. “hyper” and “drowsy” phenotypes in minks) whose traits are maintained over successive rounds of infection.

Viral strains are defined by specific polymorphisms in their respective genomes, and their existence in prion diseases was long thought to be incontrovertible evidence for the involvement of nucleic acids. However, after four decades of failed attempts to isolate any scrapie-specific genomes, strains are now thought to be caused by different PrP^{Sc} conformations which can be distinguished with conformer-sensitive fluorescent polythiophenes.

Embarrassingly for the prion field, no definitive structural evidence for these presumptions has come forward, and the "strainness" of bona fide infectious prions is still diagnosed using imperfect surrogate biomarkers such as differential resistance to disaggregation and proteolysis. By contrast, conformational heterogeneity was reported to correlate with distinct clinical phenotypes in some prionoid pathologies - although the stability of different conformations in serial transmission experiments is not yet fully established.

But how stable are prion strains across generations? RNA viruses achieve maximal fitness by creating quasispecies, clouds of variants in precarious equilibrium between adaptive mutagenesis and error catastrophe. Surprisingly, prions can also engender quasispecies whose monoclonal constituents can be isolated from cultured cells by applying various kinds of selective pressure (*15*). The structural mechanisms underlying this phenomenon are unknown and may involve conformational selection of distinct PrP^{Sc} species. The conformational selection model predicates the coexistence of multiple conformers within a single infected organism, some of which may replicate more efficiently in their host under certain environmental circumstances. The incubation time of prion infections can vary immensely between different strains, and the delay in the onset of the pathology might reflect the time needed for such selection to occur.

PrP^{Sc} conformer heterogeneity may also underlie the barriers that control interspecies prion transmission, whose strength is variable and depends both on host factors and on prion strains. While prion propagation from cows to humans results in variant Creutzfeldt-Jakob disease, sheep prions

appear to be largely innocuous to humans. This species barrier relies both on the structural diversity of the PrP^{Sc} contained in the inoculum and the PrP^{C} of the host, which cannot always interact with the misfolded conformer efficiently.

The ideas promulgated by Prusiner have undergone a remarkable metamorphosis. Templated nucleation of protein aggregates is now known to underlie not only diseases but also many physiological processes – some of which bear little resemblance to the original set of diseases that attracted Prusiner's attention. Remarkably, the structural predictions of the prion model were verified for several prionoids, but not for prions. As such, many of the questions raised by Prusiner in 1982 – prion structure, mechanism of replication, drivers of toxicity – are still open. Based on historical evidence, addressing these questions in the prion arena may, once again, provide answers that will also apply to more prevalent diseases.

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Acknowledgments

The authors thank Dr. Karl Frontzek for the micrograph of a CJD brain and Dr. Giovanni Spagnolli for the PrP^{Sc} molecular model. The Aguzzi lab is supported by the Nomis Foundation and the Swiss National Research Foundation.

Figure

Three centuries of prion science (**A**) Time line of prion-related discoveries. **(B)** Micrograph of a Creutzfeldt-Jakob brain with numerous vacuoles (spongiosis); **(C)** A polythiophene complexed with the yeast prion Het-S; (**D**) A four-rung β-solenoid structure modelling the architecture of prions.

1732 – Scrapie reported in sheep

1898 – Neuronal vacuolation recognized as a feature of scrapie

1936 – Scrapie transmissibility recognized

1959 – Similarities between scrapie, CJD and kuru reported

1982 – Prusiner isolates the scrapie agent and names it “prion”

1993 – Mice without the *Prnp* gene are resistant to prions

1996 – PrP^{c} is essential for prion neurotoxicity

2001 – Protein misfolding cyclic amplification

2007 – Spectral discrimination of prion strains

2016 – PrP^{c} controls myelin homeostasis

2019 – A plausible model of prion structure